

claimed invention, which is directed to a method for producing a synchronized population of pine somatic embryos. An agreement with respect to the claims was not reached.

The Rejection of Claims 1-13, 15-19, 21, and 23-26 Under 35 U.S.C. § 103(a) as Being Unpatentable Over U.S. Patent No. 5,294,549 (Pullman) in View of U.S. Patent No. 5,563,061 (Gupta)

Claims 1-13, 15-19, 21, and 23-26 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over U.S. Patent No. 5,294,549 (Pullman) in view of U.S. Patent No. 5,563,061 (Gupta). The Examiner admits that Pullman et al. is silent with respect to the time frame as recited in steps (b) and (c) of Claim 1. However, the Examiner has taken the view that "the amount of time the embryos are kept on the development medium is clearly a result effective parameter that a person of ordinary skill in the art would routinely optimize." The Examiner admits that none of the references teaches that the method used produces 50% or 70% of the embryo population at the same developmental stage. However, the Examiner asserts "it would be known that by using known media and other well-known medium additives, it would be obvious that one skilled in the art would have obtained 50% or 70% of the embryos population at the same developmental stage."

Applicants traverse the rejection for at least the reasons, as set forth in detail below, that the Pullman and Gupta references do not teach all the elements of the claimed invention.

Obviousness is determined by analyzing the factual inquiries set forth in *Graham v. John Deere Co.*, 383 U.S. 1, 148 U.S.P.Q. 459 (1966). *KSR* confirmed that the *Graham* factor analyses should be used in determining whether a claimed invention is obvious under 35 U.S.C. § 103(a). *KSR Int'l v. Teleflex Inc.*, 127 S. Ct. 1727, 1734 (2007). The inquiry under *Graham* includes ascertaining the differences between the prior art and the claims at issue.

The scope of Claim 1

Claim 1 recites as follows:

A method for producing a synchronized population of pine somatic embryos, the method comprising:

(a) cultivating pre-cotyledonary pine embryogenic cells in, or on a maintenance medium comprising nutrients that sustain the pine embryogenic cells;

(b) cultivating pre-cotyledonary pine embryogenic cells from step (a) for a period from one week to two weeks in, or on, a synchronization medium that comprises maltose as the principal metabolizable sugar source, an absorbent composition and at least one synchronization agent selected from the group consisting of abscisic acid and a gibberellin, wherein the absorbent composition and the at least one synchronization agent are present at a concentration effective to produce a synchronized population of pre-cotyledonary pine somatic embryos wherein at least 50% of the pre-cotyledonary pine somatic embryos in the synchronized population are at the same developmental stage; and

(c) transferring the synchronized population of pre-cotyledonary pine somatic embryos from step (b) to a development medium and culturing the pre-cotyledonary pine somatic embryos for a period from 9 to 14 weeks to produce a synchronized population of cotyledonary pine somatic embryos. (Emphasis added).

The differences between U.S. Patent No. 5,294,549 (Pullman) and Claim 1

It is noted that Pullman does not teach or suggest cultivating pre-cotyledonary pine embryogenic cells for a period from one week to two weeks in, or on a synchronization medium, as recited in Claim 1 step (b). In contrast to the claimed invention, Pullman discloses a culturing step referred to as "singulation" for Douglas-fir. See Pullman et al. at Col. 8, lines 18–21. Pullman et al. teaches the transfer of pre-cotyledonary Douglas-fir somatic embryos from a maintenance medium to a singulation medium for at least three weeks, followed by transfer to a development medium. As described in Examples 1–7, which are directed to methods for improving Douglas-fir embryo development, "Late stage Douglas-fir proembryos were singulated in a three step liquid shake culture as outlined above." Example 2 at Col. 15, line 68, to Col. 16, line 2. As described in Example 1, a preferred schedule for the singulation step in Douglas-fir is "one week on a medium containing 10mg/L ABA, a second week on a medium containing 5mg/L ABA, and a third week on a medium also with 5mg/L ABA." Col. 15, lines 10–27.

It is further noted that Pullman does not teach or suggest the use of a medium that comprises maltose as the principal metabolizable sugar source, an absorbent composition and at least one synchronization agent selected from the group consisting of abscisic acid and a gibberellin, as claimed.

The differences between Gupta and Claim 1

It is noted that Gupta does not teach or suggest cultivating pre-cotyledonary pine embryogenic cells for a period from one week to two weeks in, or on a synchronization medium, as recited in Claim 1 step (b). Rather, in contrast to the claimed invention, Gupta et al. teaches that Douglas-fir requires an intermediate singulation culturing step between early stage embryo

growth and the final development stage due to the formation of tight clusters of embryos. As described in Gupta, singulation is carried out in a series of liquid shake cultures lacking auxins and cytokinins but which have exogenous abscisic acid added as a necessary new hormone. Gupta at Col. 8, lines 4-9.

All of the claimed elements are not found in the cited references

In order to establish a *prima facie* case of obviousness, all of the claimed elements must be found in the prior art. See M.P.E.P. § 2143.

As discussed supra, both Pullman and Gupta teach a culturing step referred to as "singulation" for Douglas-fir in which pre-cotyledonary Douglas-fir somatic embryos from a maintenance medium to a singulation medium for at least three weeks, followed by transfer to a development medium. Neither Pullman or Gupta teach or provide any suggestion regarding culturing pre-cotyledonary pine Embryogeny cells in synchronization medium for from one to two weeks, as recited in step (b) of Claim 1.

Accordingly, because neither of the cited references provides any teaching regarding the synchronization of pre-cotyledonary pine embryogenic cells, and in particular, the cultivation of pre-cotyledonary pine embryogenic cells for a period of one to two weeks in synchronization medium as claimed, the cited references alone or in combination do not teach or suggest every element of Claim 1.

No motivation to modify the teachings of Pullman and/or Gupta to arrive at the claimed invention

There is no suggestion or motivation provided in either Pullman or Gupta to modify the teachings of the cited references, which are both directed to singulation in Douglas-fir, in order

to arrive at the claimed invention, which is directed to synchronization of pine pre-cotyledonary embryos. As noted *supra*, the step of singulation is carried out during Douglas-fir embryogenesis due to the formation of tight clusters of Douglas-fir embryos. As described in the cited references, the step of singulation in Douglas-fir is carried out for at least three weeks in a series of liquid shake cultures. See Gupta at Col. 8, lines 4-9; and Pullman et al. at Col. 8, lines 18-21. There is no suggestion or motivation provided in either reference to modify the teachings to reduce the time of incubation in singulation medium to 1 to 2 weeks, as claimed, because the proposed modification would likely render the methods of the cited references inoperable, or at least less efficacious, for their intended purpose of singulation.

Moreover, it is further noted that the Examiner admits that neither Pullman nor Gupta teach or remotely suggest synchronization of embryos. As described in the present specification, the claimed invention is based on the discovery by the present inventors that culturing pine embryos in a synchronization medium that comprises maltose as the principle metabolizable sugar source, an absorbent composition (e.g., activated charcoal) and at least one of abscisic acid and a gibberellin for one to two weeks prior to incubation in development media inhibited precocious embryo development and greening, while promoting synchronization of the cultures, thereby resulting in embryos very uniform in size in comparison to control cultures. Specification at page 19, lines 19-31; page 16, lines 26-30; and Tables 1 and 2. As further described in the instant specification, it was experimentally determined that in the absence of the step of culturing in a synchronization medium (i.e., control cultures grown in maintenance medium and directly transferred to development medium, similar to Examples 8 and 9 of Pullman et al.), the resulting cultures were not synchronized, and contained embryos that were cleaving, growing, and forming embryo suspensor masses, with embryos seen in many different stages. Specification at page 19, lines 1-5.

Accordingly, applicants submit that the Examiner has not established a *prima facie* case of obviousness because the Pullman and Gupta references, alone or in combination do not teach or suggest all the elements of amended Claim 1. Accordingly, Claim 1 and dependent Claims 2–13, 17–19, 21, and 23–26 are not obvious over the cited references. Withdrawal of the rejection is respectfully requested.

Conclusion

Applicants believe that Claims 1–13, 17–19, 21 and 23–26 are in condition for allowance. Reconsideration and favorable action is requested. The Examiner is further requested to contact the applicants' representative at the number set forth below to discuss any issues that may facilitate prosecution of this application.

Respectfully submitted,

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